expressed in the tumor and that the difference in toxicity between prodrug and drug is a hundred-fold or more then, once a candidate enzyme has been identified, many classes of anti-cancer agent can often be derivatized to form appropriate prodrugs.

The presently claimed method is directed to a novel prodrug activation system in which the enzyme may be endogenous to human tumor cells. The co-substrate can be administered using standard methods of administration for most drugs. See, for example, pages 47-52; also Figures 11 and 12, showing conversion of the prodrug in the presence of co-substrate to a cytotoxic compound, and Figures 15-18, showing the safety and non-toxicity of administration of the NRH co-substrate.

CB 1954 is an antitumor prodrug that is activated in certain rat tumors via its 4-hydroxylamine derivative to a potent bifunctional alkylating agent. Human tumor cells are normally unable to efficiently catalyze the conversion of CB 1954 to a cytotoxic agent via the human enzyme NQO1 which uses endogenous NAD(P)H as a co-substrate to reduce CB 1954. Human NQO1 does not appreciably convert CB 1954 into its cytotoxic form. However, another human enzyme has been discovered that can activate CB 1954, and it has been shown to be commonly present in human tumor cells. The enzyme is NQO2, but its activity is normally latent, and a nonbiogenic exogenous co-substrate such as NRH is required for enzymatic activity (see, for example, page 8, lines 18-21). This ternary system, including the cells to be killed, the enzyme, NQO2, that the cells express, the prodrug CB 1954, and the exogenous co-substrate for the enzyme, NRH, is inactive if any one of the compounds is absent.

CB 1954 is a *proven* anti-tumor agent as defined by *in vivo* work in rats, in which CB 1954 is activated by the rat enzyme NQO1 (page 5, line 26 to page 7, line 16). Although NQO1 is present in human cells and is therefore an exploitable enzyme for inducing selective cytotoxicity as its levels are significantly raised (compared with the surrounding normal tissue) in tumor tissue, the human form of NQO1 metabolizes CB 1954 much less efficiently than rat NQO1 (Wu *et al.*, *Arch. Biochem. Biophys.*, 347:221-228, 1997). Thus, even those cells that are high in human NQO1 are insensitive to CB 1954, because there is inefficient conversion of CB 1954 to its toxic form. The catalytic difference between the two forms of the enzyme NQO1 (rat and human) is mainly accounted for by a single amino acid change at residue 104 (tyrosine in the rat enzyme and glutamine in the human enzyme). NQO2, which was identified on the basis of its homology to DT-diaphorase (NQO1), reacts with CB 1954 to produce the *identical* cytotoxic product as that produced by rat NQO1.

Accordingly, once one shows that in the presence of the appropriate exogenous co-substrate, NRH, the prodrug CB 1954 is converted by NQO2, an enzyme expressed in human tumor cells, into a cytotoxic drug, those skilled in the art would expect the claimed method to be effective, and would be enabled to use the method.

The enclosed Declaration under 37 C.F.R. 1.132 by Professor Richard Knox, provides further evidence of why one can extrapolate results obtained *in vitro* and in rat studies to humans.

Rejection Under 35 U.S.C. § 112, first paragraph

In the Office Action issued as Final in the parent application (U.S.S.N. 09/445,865), claims 29, 31-33, 40 and 41 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner did not argue that one could not administer either the claimed prodrug CB 1954, nor the co-substrate, NRH, to a patient in need of treatment. The Examiner has provided no support that one could not practice the claimed method. Rather, the Examiner actually made a utility rejection, under the guise of a section 112, lack of enablement, heading, knowing that the standard for utility had been met.

The standard under 35 U.S.C. 101, utility, has most recently been reviewed in the MPEP as follows. M.P.E.P. § 2107.01 clearly states that deficiencies under the "useful invention" requirement of 35 U.S.C. § 101 can occur when an applicant fails to identify any specific and substantial utility for the invention or fails to disclose enough information about the invention to make its usefulness immediately apparent to those familiar with the field of the invention. See *Brenner v. Manson*, 383 U.S. 519, 148 USPQ 689 (1966); *In re Ziegler*, 992 F.2d 1197, 26 USPQ2d 1600 (Fed. Cir. 1993). A second type of deficiency arises in the rare instance where an assertion of specific and substantial utility for the invention made by an applicant is not credible.

Therefore, absent a showing that one skilled in the art would not expect the claimed method to be efficacious, the examiner has failed to meet the burden under either 35 USC section 101 or 112, enablement. The examiner has provided no such support, only allegations that the

examples, because they do not show efficacy in humans, do not demonstrate that the claimed method is enabled.

Analysis of Claims 29, 31-33, and 40 under 35 U.S.C. § 112, first paragraph or under 101.

CB 1954 may be converted into a difunctional alkylating agent that has been demonstrated to have a dramatic and highly selective activity against the rat Walker 256 tumor, expressing NQO1, which is known to convert CB 1954 into a cytotoxic compound. These studies are outlined in the specification (see page 6 and Figure 1).

CB 1954 is not effective as a cytotoxic agent in the presence of the *human* form of NQO1. *Human* NQO1 does not appreciably convert CB 1954 into its cytotoxic form. Therefore, human tumors are not inherently resistant to CB 1954 in its cytotoxic form (page 6, line 24 to page 7, line 4 of the present specification) but are not killed by CB 1954 alone due to the inefficiency of the endogenous NQO1 enzyme. However, one of ordinary skill in the art will appreciate that the initial activation of CB 1954 by human NQO2, in the presence of an *exogenous* co-substrate (i.e. NRH), leads to a hydroxylamine intermediate (see Figure 3 of the present specification) which is identical to the hydroxylamine intermediate produced by the activation of NQO1, in the presence of an *endogenous* co-substrate (i.e. NAD(P)H), though not by the same mechanism (see Figure 1 of the present specification). Therefore, one skilled in the art would have every expectation that administration of the prodrug and the exogenous co-substrate NRH would lead to production of cytotoxic compound.

It is well accepted in the art that reduction of CB 1954 to its 4-hydroxylamine derivative can lead to an anti-tumor effect irrespective of the enzyme involved (page 6, lines 12-22 of the present application). Indeed, U.S. Patent No. 5,780,585 and U.S. Patent No. 5,958,682 referenced above teach the use of an *E. coli* nitroreductase enzyme to activate CB 1954 for the treatment of human tumors. Nitroreductase has been used in gene targeting assays (gene directed enzyme prodrug therapy and antibody directed enzyme prodrug therapy) because it reduces CB 1954 much more rapidly than rat NQO1, although forming an equal mixture of the 2- and 4-hydroxylamines (the 2-hydroxylamine is less toxic than the corresponding 4-derivative, but still more cytotoxic than CB 1954 itself). NQO2 resembles rat NQO1 in that, unlike the bacterial enzyme, it forms only the more cytotoxic 4-hydroxylamine reduction product of CB 1954. Rat NQO1, in the presence of a co-substrate, activates CB 1954 *in vivo* to produce an effective anti-tumor agent. One of ordinary skill in the art would realize that activation of CB 1954 by administration of an exogenous co-substrate with the prodrug, as presently claimed, would be effective in the treatment of human tumors.

Example 8 of the present specification demonstrates the potentiation of CB 1954 in human glioblastoma cells in the presence of NQO2 co-substrates. These glioblastoma cells were *not* transfected with NQO2 in these experiments. The prodrug CB 1954 was exposed to levels of NQO2 occurring *naturally* in the cell line, in combination with exogenous substrate.

It is acknowledged that human NQO2 is *not* the same enzyme as NQO1. If human cells expressed an NQO1 that was able to convert the prodrug to a cytotoxic compound in human cells, there would be no need of the claimed method. However, what applicants have discovered

is that human cells express a different enzyme, NQO2, which, when an exogenous co-substrate is administered to the cells, is effective in converting the prodrug to a cytotoxic compound, the same cytotoxic compound as shown to be produced by the action of the rat NQO1 enzyme utilizing endogenous substrate. See Table 1 on page 57 of the originally filed specification. Table 1 demonstrates that human NQO2 in the presence of an NRH co-substrate reduces CB 1954 six hundred times faster than human NQO1 and a hundred times faster than rat NQO1. The actual cytotoxic compound responsible for the cytotoxic effect *is the same* as that shown to elicit the effect in response to rat NQO1.

The applicants have not extrapolated the efficacy of the resultant cytotoxic compound from *in vitro* studies. The applicants submit that in paper 12 (Response and amendment to the Office Action mailed on April 10, 2001), page 5 (second paragraph), the statement referring to examples being used "as a way to illustrate that the administration of co-substrates *to living cells* is achievable...." (emphasis added), does not reflect upon the administration of co-substrates *in vitro*. The efficacy of the resultant cytotoxic compound (i.e. CB 1954), has been demonstrated not only in cells *in vitro* but also based on the above-mentioned rat *in vivo* studies. Accordingly, one skilled in the art would be enabled to use the claimed method, with an expectation that it would be effective in producing a cytotoxic compound from the prodrug CB 1954.

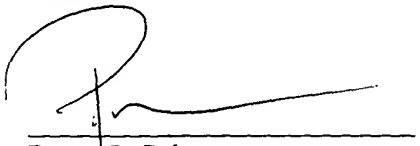
The applicants have shown that human enzyme NQO2 exists and that in the presence of an exogenous NRH co-substrate can convert the prodrug CB 1954 to a cytotoxic compound for use in a simple but selective antitumor therapy. It is clear that the mechanism of action of CB 1954 is fully understood and it is accepted that its lack of activity against human tumors is due to

the very inefficient conversion of CB 1954 to its active form by the human enzyme NQO1. The applicants have described a method of overcoming this fundamental limitation - add an exogenous co-substrate for a separate and distinct enzyme, NQO2, which is then able to convert the CB 1954 to a cytotoxic compound. There is no reason to suggest that CB 1954, activated using the disclosed method, should be less effective as an anti-tumor agent than CB 1954 activated by any other method (for example, as in rats where it is a proven anti-tumor agent).

As provided in the foregoing discussion, four salient points need to be reiterated: (1) the cytotoxic form of CB 1954 is identical regardless of the converting enzyme; (2) the 4-hydroxylamine intermediate that is formed during this conversion reaction is identical in reactions governed by NQO1 or NQO2: (3) rat NQO1, in the presence of an endogenous co-substrate, activates CB 1954 *in vivo* to produce an effective anti-tumor agent (reviewed in Knox *et al.*, *Cancer Metastasis Rev.*, 1993); and (4) NQO2 in the presence of an exogenous co-substrate (NRH) activated CB 1954 *in vitro* to selectively kill human tumor cells. Therefore one skilled in the art would predict that the claimed method would be effective in treating humans.

Allowance of claims 1-29 and 31-41 is respectfully solicited.

Respectfully submitted,



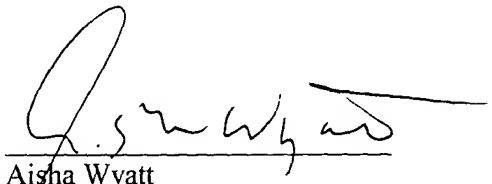
Patrea L. Pabst
Reg. No. 31,284

Date: August 16, 2002

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.



Aisha Wyatt

Date: August 16, 2002

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. A compound comprising a target cell-specific portion and human NAD(P)H:quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof which has substantially the same activity as NQO2 towards a given prodrug, or a polynucleotide encoding said NQO2 or said variant or fragment or fusion or derivative, wherein the prodrug is CB 1954 and analogs thereof.

2. (Amended) A compound according to claim 1 comprising a target cell-specific portion and human NAD(P)H:quinone reductase 2 (NQO2).

3. (Amended) A compound according to claim 1 wherein the target cell-specific portion is tumour cell-specific.

4. (Amended) A compound according to claim 1 wherein the target cell-specific portion comprises an antibody or fragment or derivative.

5. (Amended) A compound according to claim 1 wherein the target cell-specific portion comprises a macromolecule.

6. (Amended) A compound according to claim 1 wherein the human NAD(P)H:quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof is capable of being located substantially inside or following expression of the polynucleotide is located substantially inside the target cell.

7. (Amended) A compound according to claim 1 comprising means for delivering said polynucleotide to said target cell.

8. A recombinant polynucleotide comprising a target cell-specific promoter operably linked to a polynucleotide encoding human NAD(P)(H):quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof which has substantially the same activity as NQO2 towards a given prodrug.

9. (Amended) A recombinant polynucleotide according to claim 8 wherein said promoter is tumour cell-specific.

10. (Amended) A recombinant polynucleotide according to claim 8 comprising a polynucleotide encoding NQO2.

11. (Amended) A recombinant polynucleotide according to claim 8 which is capable, following expression in a target cell, of providing the NQO2 or a variant or fragment or fusion or derivative thereof located substantially inside the target cell.

12. (Amended) A compound according to claim 1 wherein said polynucleotide is the recombinant polynucleotide of claim 8.

13. (Amended) A therapeutic system comprising a compound according to claim 1, or a polynucleotide according to claim 8 and a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

14. (Amended) A system according to claim 13 wherein the prodrug is CB 1954 or an analogue thereof.

15. (Amended) A system according to claim 14 wherein the prodrug is CB 1954.

16. (Amended) A system according to claim 13 further comprising a cosubstrate for NQO2.

17. (Amended) A system according to claim 16 wherein the cosubstrate is nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

18. (Amended) A method of treating a patient with a target cell to be destroyed the method comprising (a) administering to the patient a compound according to claim 1, or a recombinant polynucleotide according to claim 8; (b) allowing the NQO2 or a variant or fragment or fusion or derivative thereof to localize at, or be expressed in, the target cell; and (c) administering a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

19. (Amended) A method according to claim 18 wherein the patient has a tumour to be treated.

20. (Amended) A method according to claim 18 wherein the prodrug is CB 1954 or an analogue thereof.

21. (Amended) A method according to claim 20 wherein the prodrug is CB 1954.

22. (Amended) A method according to claim 18 the method further comprising administering to the patient a cosubstrate for NQO2.

23. (Amended) A method according to claim 22 wherein the cosubstrate is nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

24. (Amended) A compound according to claim 1, or a recombinant polynucleotide according to claim 8, for use in medicine.

25. (Amended) Use of a compound according to claim 1, or a recombinant polynucleotide according to claim 8, in the manufacture of a medicament for treating a patient with a target cell to be destroyed.

26. (Amended) Use as defined in claim 25 wherein the patient has been, is being or will be administered a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

27. (Amended) Use of a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 in the manufacture of a medicament for treating a patient with a target cell to be destroyed wherein the patient has been, is being or will be administered a compound according to claim 1, or a recombinant polynucleotide according to claim 8.

28. (Amended) Use as defined in claim 27 wherein the patient has a tumour to be treated.

29. (Twice Amended) A method of treating a human patient with a target cell to be destroyed wherein the target cell expresses NQO2 the method comprising administering to the patient a prodrug which is converted to a cytotoxic drug by the action of NQO2 and nicotinamide

riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2, wherein the prodrug is CB 1954.

Please cancel claim 30.

31. (Amended) The method of claim 29 wherein the analogue of NRH is able to permeate the target cell membrane.

32. (Amended) The method of claim 29 wherein the target cell is a tumour.

33. (Amended) The method of claim 29 the method further comprising determining, before administering the prodrug or NRH or an analogue thereof, whether the target cell to be treated expresses NQO2.

34. A therapeutic system comprising a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 and nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

35. Nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2 for use in medicine.

36. Use of nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2 in the manufacture of a medicament for treating a human patient with a target cell to be destroyed.

37. (Amended) Use as defined in claim 36 wherein the patient has been, is being or will be administered a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

38. Use of a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 in the manufacture of a medicament for treating a human patient with a target cell to be destroyed wherein the patient has been, is being or will be administered NRH or an analogue thereof which can pass reducing equivalents to NQO2.

39. A kit of parts comprising a means for determining whether a target cell to be treated expresses NQO2 and NRH or an analogue thereof which can pass reducing equivalents to NQO2.

40. The method of claim 29 wherein the patient has cancer.

41. (New) The method of claim 29, wherein the analogue of NRH is 1-(carboxamidomethyl)-dihydronicotinamide.

U.S.S.N. *
Filed: *
MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

Clean Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. A compound comprising a target cell-specific portion and human NAD(P)H:quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof which has substantially the same activity as NQO2 towards a given prodrug, or a polynucleotide encoding said NQO2 or said variant or fragment or fusion or derivative, wherein the prodrug is CB 1954 and analogs thereof.

2. (Amended) A compound according to claim 1 comprising a target cell-specific portion and human NAD(P)H:quinone reductase 2 (NQO2).

3. (Amended) A compound according to claim 1 wherein the target cell-specific portion is tumour cell-specific.

4. (Amended) A compound according to claim 1 wherein the target cell-specific portion comprises an antibody or fragment or derivative.

5. (Amended) A compound according to claim 1 wherein the target cell-specific portion comprises a macromolecule.

6. (Amended) A compound according to claim 1 wherein the human NAD(P)H:quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof is capable of being located substantially inside or following expression of the polynucleotide is located substantially inside the target cell.

7. (Amended) A compound according to claim 1 comprising means for delivering said polynucleotide to said target cell.

8. A recombinant polynucleotide comprising a target cell-specific promoter operably linked to a polynucleotide encoding human NAD(P)(H):quinone reductase 2 (NQO2) or a variant or fragment or fusion or derivative thereof which has substantially the same activity as NQO2 towards a given prodrug.

9. (Amended) A recombinant polynucleotide according to claim 8 wherein said promoter is tumour cell-specific.

10. (Amended) A recombinant polynucleotide according to claim 8 comprising a polynucleotide encoding NQO2.

11. (Amended) A recombinant polynucleotide according to claim 8 which is capable, following expression in a target cell, of providing the NQO2 or a variant or fragment or fusion or derivative thereof located substantially inside the target cell.

12. (Amended) A compound according to claim 1 wherein said polynucleotide is the recombinant polynucleotide of claim 8.

13. (Amended) A therapeutic system comprising a compound according to claim 1, or a polynucleotide according to claim 8 and a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

14. (Amended) A system according to claim 13 wherein the prodrug is CB 1954 or an analogue thereof.

15. (Amended) A system according to claim 14 wherein the prodrug is CB 1954.

16. (Amended) A system according to claim 13 further comprising a cosubstrate for NQO2.

17. (Amended) A system according to claim 16 wherein the cosubstrate is nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

18. (Amended) A method of treating a patient with a target cell to be destroyed the method comprising (a) administering to the patient a compound according to claim 1, or a recombinant polynucleotide according to claim 8; (b) allowing the NQO2 or a variant or fragment or fusion or derivative thereof to localize at, or be expressed in, the target cell; and (c) administering a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

19. (Amended) A method according to claim 18 wherein the patient has a tumour to be treated.

20. (Amended) A method according to claim 18 wherein the prodrug is CB 1954 or an analogue thereof.

21. (Amended) A method according to claim 20 wherein the prodrug is CB 1954.

22. (Amended) A method according to claim 18 the method further comprising administering to the patient a cosubstrate for NQO2.

23. (Amended) A method according to claim 22 wherein the cosubstrate is nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

24. (Amended) A compound according to claim 1, or a recombinant polynucleotide according to claim 8, for use in medicine.

25. (Amended) Use of a compound according to claim 1, or a recombinant polynucleotide according to claim 8, in the manufacture of a medicament for treating a patient with a target cell to be destroyed.

26. (Amended) Use as defined in claim 25 wherein the patient has been, is being or will be administered a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

27. (Amended) Use of a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 in the manufacture of a medicament for treating a patient with a target cell to be destroyed wherein the patient has been, is being or will be administered a compound according to claim 1, or a recombinant polynucleotide according to claim 8.

28. (Amended) Use as defined in claim 27 wherein the patient has a tumour to be treated.

29. (Twice Amended) A method of treating a human patient with a target cell to be destroyed wherein the target cell expresses NQO2 the method comprising administering to the patient a prodrug which is converted to a cytotoxic drug by the action of NQO2 and nicotinamide

riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to $NQO2_2$ wherein the prodrug is CB 1954.

31. (Amended) The method of claim 29 wherein the analogue of NRH is able to permeate the target cell membrane.

32. (Amended) The method of claim 29 wherein the target cell is a tumour.

33. (Amended) The method of claim 29 the method further comprising determining, before administering the prodrug or NRH or an analogue thereof, whether the target cell to be treated expresses NQO2.

34. A therapeutic system comprising a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 and nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2.

35. Nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2 for use in medicine.

36. Use of nicotinamide riboside (reduced) (NRH) or an analogue thereof which can pass reducing equivalents to NQO2 in the manufacture of a medicament for treating a human patient with a target cell to be destroyed.

37. (Amended) Use as defined in claim 36 wherein the patient has been, is being or will be administered a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2.

38. Use of a prodrug which is converted to a substantially cytotoxic drug by the action of NQO2 in the manufacture of a medicament for treating a human patient with a target cell to be destroyed wherein the patient has been, is being or will be administered NRH or an analogue thereof which can pass reducing equivalents to NQO2.

39. A kit of parts comprising a means for determining whether a target cell to be treated expresses NQO2 and NRH or an analogue thereof which can pass reducing equivalents to NQO2.

40. The method of claim 29 wherein the patient has cancer.

41. (New) The method of claim 29, wherein the analogue of NRH is 1-(carboxamidomethyl)-dihydronicotinamide.

ATL1 #524202 v1